AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 5, lines 8-19, and replace it with the following paragraph:

Figure 1 represents (a) Online SAGE Tag to Gene Mapping analysis demonstrating the frequency of the Hs.111779 tag (ATGTGAAGAG **(SEQ ID NO: 13)**) corresponding to the *SPARC* gene in 8 pancreatic SAGE libraries derived from short-term cultures of normal pancreatic ductal epithelial cells (H126 and HX), pancreatic cancer cell lines (CAPAN1, CAPAN2, HS766T, and Panc1), and primary pancreatic adenocarcinoma tissue (Panc 91-16113 and Panc 96-6252); (b) Gene expression analysis of *SPARC* by oligonucleotide microarrays in two frozen tissue samples of normal pancreatic ductal epithelial cells selectively microdissected by LCM, a non-neoplastic pancreatic epithelial cell line (HPDE), and 5 pancreatic cancer cell lines (AsPC1, CFPAC1, Hs766T, MiaPaCa2, and Panc1); (c) Reverse transcription-PCR analysis of SPARC in a non-neoplastic pancreatic duct epithelial cell line (HPDE), primary fibroblasts derived from pancreatic cancer, and 17 pancreatic cancer cell lines; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serves as an RNA control.

Please delete the paragraphs on page 6, lines 22-27, and replace them with the following paragraphs:

Figure 7 represents the nucleic acid sequence **(SEQ ID NO: 8)** for the bisulfite sequencing primers; forward (SEQ ID NO: 2) and reverse (SEQ ID NO: 3).

Figure 8 represents the methylation specific PCR primers: Unmethylated **(DNA sequence disclosed as SEQ ID NO: 9)**, forward (SEQ ID NO: 4) and reverse (SEQ ID NO: 5); and Methylated **(DNA sequence disclosed as SEQ ID NO: 10)**, forward (SEQ ID NO: 6) and reverse (SEQ ID NO: 7).

Please delete the paragraphs on page 32, line 7 to page 33, line 3 and replace them with the following paragraphs:

Total RNA was isolated from cultured cells or frozen tissues using TRIZOL reagent (Invitrogen, Carlsbad, CA). First- and second-stranded cDNA was synthesized from 10 □g of total RNA using T7-$(dT)_{24}$ primer **(SEQ ID NO: 14)** (Genset Corp., South La Jolla, CA) and SuperScript Choice system (Invitrogen). Labeled cRNA was synthesized from the purified cDNA by in vitro transcription (IVT) reaction using the BioArray HighYield RNA Transcript Labeling Kit (Enzo Diagnostics, Inc., Farmingdale, NY) at 37°C for 6 hours, and was purified using RNeasy Mini Kit (QIAGEN, Valencia, CA). The cRNA was fragmented at 94°C for 35 minutes in a fragmentation buffer (40 mmol/L Tris-acetate (pH 8.1), 100 mmol/L potassium acetate, 30 mmol/L magnesium acetate). The fragmented cRNA was then hybridized to the Human Genome U133A chips (Affymetrix, Santa Clara, CA) with 18,462 unique gene/EST transcripts at 45°C for 16 hours. The washing and staining procedure was performed in the Affymetrix Fluidics Station according to the manufacturer's instructions. The probes were then scanned using a laser scanner, and signal intensity for each transcript (background-subtracted and adjusted for noise) and detection call (present, absent, or marginal) were determined using Microarray Suite Software 5.0 (Affymetrix).

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

Four μg of total RNA was reverse-transcribed using Superscript II (Invitrogen). The SPARC RT-PCR reaction was performed under the condition as follow: 95°C for 5 minutes; then 28 cycles of 95°C for 20 seconds, 63°C for 20 seconds, and 72°C for 20 seconds; and a final extension of 4 minutes at 72°C. Primer sequences were 5'-AAG ATC CAT GAG AAT GAG AAG-3' (forward) **(SEQ ID NO: 11)** and 5'-AAA AGC GGG TGG TGC AAT G-3' (reverse) **(SEQ ID NO: 12)**. To check the integrity of mRNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also amplified in the same PCR condition. For semiquantitative analysis, the RT-PCR was performed with primers for SPARC and GAPDH in duplex reactions, and range of linear amplification for both genes was examined with serial PCR cycles to determine the

optimal cycle. The relative intensity of SPARC mRNA expression was then corrected for variable RNA recovery using the corresponding GAPDH mRNA measurement as a surrogate for total mRNA.

Please delete the paragraph on page 33, lines 18-28, and replace it with the following paragraph:

Methylation status of the *SPARC* gene was determined by MSP as described previously (Herman *et al.*, 1996). Briefly, 1 µg of genomic DNA was treated with sodium bisulfite for 16 hours at 50°C. After purification, 1 µl of the bisulfite-treated DNA was amplified using primers specific for either the methylated or for the unmethylated DNA under the conditions as follows: 95°C for 5 minutes; then 40 cycles of 95°C for 20 seconds, 62°C for 20 seconds, and 72°C for 30 seconds; and a final extension of 4 minutes at 72°C. Primer sequences were TTT TTT AGA TTG TTT GGA GAG TG (forward) **(SEQ ID NO: 4)** and AAC TAA CAA CAT AAA CAA AAA TAT C (reverse) **(SEQ ID NO: 5)** for unmethylated reactions (132bp), and GAG AGC GCG TTT TGT TTG TC (forward) **(SEQ ID NO: 6)** and AAC GAC GTA AAC GAA AAT ATC G (reverse) **(SEQ ID NO: 7)** for methylated reactions (112bp). Five µl of each PCR product were loaded onto 3% agarose gels and visualized by ethidium bromide staining.

Please delete the paragraph on page 35, lines 8-21, and replace it with the following paragraph:

Oligonucleotide microarrays have been used to identify genes that are induced 5-fold or greater by treatment of pancreatic cancer cells with 5Aza-dC (Sato *et al.*, manuscript submitted). *SPARC* was one of the genes we identified using this approach. We therefore analyzed the gene expression and methylation status of the *SPARC* gene in pancreatic cancer. First, we searched an online SAGE database (http://www.ncbi.nlm.nih.gov/SAGE/) to determine the gene expression patterns of *SPARC* in short-term cultures of normal pancreatic ductal epithelium, pancreatic cancer cell lines, and primary pancreatic cancer tissues. The SAGE Tag to Gene Mapping analysis showed that the Hs.111779 tag (ATGTGAAGAG **(SEQ ID NO: 13)**)

corresponding to the *SPARC* gene was present in both of two libraries from normal pancreatic duct epithelial cell cultures (H126 and HX), whereas the *SPARC* tag was not identified in 3 of 4 pancreatic cancer cell lines (Figure 1A). By contrast, the *SPARC* tag was detected at high levels in two primary pancreatic adenocarcinoma tissues (Panc 91-16113 and Panc 96-6252), suggesting that this gene may be an "invasion-specific gene" a gene whose expression is specifically identified in tissue specimens of invasive pancreatic cancer but not in passaged pancreatic cancer cell lines (Ryu *et al.*, 2001).